

Phase separation across membranes and condensates in cell organization and function

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Phase separation is a fundamental principle of cellular organization that typically leads to two coexisting phases: a dense one, where intermolecular interactions are stronger and molecules are more tightly packed, and a dilute, less packed phase with weaker interactions and lower molecular concentration. This process drives the formation of both lateral membrane domains (rafts) and liquid-like protein and nucleic acid condensates. This Review explores the dynamic interplay between biomolecular condensates and membrane lipid domains, and how phase separation occurs in the three-dimensional (bulk) cellular interior and at membrane interfaces. We examine how membranes act as platforms influencing condensate formation and function, and conversely, how condensates modulate membrane properties and organization. By highlighting diverse examples from cell signaling, tight junction assembly, and stress responses, we emphasize how these coupled interactions are crucial for cellular organization, function, and fitness.

Introduction

Phase separation, a fundamental concept in physical chemistry, describes the demixing of components into distinct phases under specific thermodynamic conditions like temperature, concentration, and molecular interactions. Biological membranes are intricate assemblies, buzzing with hundreds of different lipids and proteins that engage in a wide range of specific and nonspecific interactions. Because these components do not mix ideally but instead exhibit preferential, energetically favorable interactions, membranes deviate from ideal solution behavior. These non-ideal interactions can promote lateral heterogeneity and, under certain conditions, drive two-dimensional (2D) membrane phase separation, a fundamental phenomenon of cellular organization. A similar principle applies to the bulk cytoplasm: the sheer abundance of thousands of different macromolecules and their interactions prevents ideal mixing. Instead, these components can undergo three-dimensional (3D) phase separation, forming specialized liquid-like compartments known as membraneless organelles or biomolecular condensates¹. The discovery of these organelles has revolutionized cell biology, yet we are still learning how their unique material properties dictate functions² and how their composition can be tuned to modulate their behavior³.

Increasing evidence indicates a crucial interplay between membrane-bound and membraneless organelles⁴. These organelles not only interact with, but can also remodel one another, altering each other's physical properties³. This interaction is vital for a diverse range of cellular processes, including signal transduction⁵, immune response⁶, vacuolar morphogenesis in plants⁷, pre- and postsynapse formation^{8,9}, and response to stress¹⁰. However, this burgeoning field presents challenges. Both membranes and condensates are multimolecular, self-organizing structures that exhibit emergent properties arising from the collective interactions of their individual components. Consequently, a multi-scale approach is essential for comprehensive understanding, spanning from the molecular scale to the mesoscopic range (hundreds of nanometers to tens of micrometers). The development of super-resolution techniques combined with spectroscopic methods is instrumental in bridging this gap, enabling the analysis of various properties of condensates and membranes and disentangling their complex interactions¹¹⁻¹⁴. In addition, model systems are indispensable for overcoming cellular complexity, as they allow precise control of key parameters to obtain reliable measurements of the physicochemical processes governing condensate–membrane interactions⁴.

Although the seminal work by Brangwynne et al. describing P-bodies in *Caenorhabditis elegans* as membraneless (3D) organelles in 2009 (ref. ¹⁵) is widely recognized for establishing biomolecular condensates as an emerging field, earlier reports had already hinted at their existence. For instance, electron microscopy studies dating back to the 1980s, even predating the terms “membraneless organelles” or “biomolecular condensates”, showed evidence of storage

protein condensates (referred to as “protein masses”) **wetting [G]** and remodeling the tonoplast (vacuolar membrane) in plants¹⁶. However, these early observations were not interpreted through the lens of phase separation. The work of Brangwynne and colleagues already highlighted their interaction with the nuclear membrane¹⁵. Shortly after, condensates were observed at membrane surfaces as 2D clusters of receptors¹⁷⁻¹⁹. For decades, lateral phase separation and “**raft [G]**” formation in membranes (where domains arise as result of liquid–liquid phase separation of the lipids) have been in the spotlight of membrane cell biology and biophysics²⁰⁻²². Now, a new level of complexity is added: liquid protein phases can also form domains at membranes, and even coupling mechanisms between lipid and protein phases have been observed^{5,6,11,23-26}.

In this Review, we describe lipid phase separation at membranes, introducing the fundamental terminology and tools employed to understand phase separation in general. Afterwards, we discuss condensate formation and properties, highlighting techniques that provide information across different scales. Finally, we discuss the intricate interaction between condensates and membranes, showing examples for both 2D and 3D condensates. Throughout the Review, we emphasize the knowledge gained from various model systems and conclude with selected examples of condensate–membrane interactions in cell biology.

Lateral phase separation in membranes

Membranes are more than just cellular barriers; they are active platforms for vital processes. This section explores how lateral phase separation drives the formation of membrane domains or rafts in both minimal systems and cells, intimately impacting protein organization, membrane properties and protein function.

Phase diagrams of model and complex systems

In essence, phase separation describes the thermodynamically-driven demixing of components into distinct phases, determined by parameters including temperature, composition, and intermolecular interactions. In biological systems, this phenomenon takes on new levels of complexity due to the great diversity of components, and their highly dynamic behavior. **Phase diagrams [G]** are crucial tools for mapping phase separation, illustrating the conditions under which different phases coexist and transform. For instance, mixing lipids with high and low melting temperatures often leads to demixing, displaying regions of phase coexistence, such as gel–solid (So) and liquid–crystalline (L α) phases²⁷ (see Figure 1a for an example of a binary phase diagram and Box 1).

The presence of sterols significantly influences membrane phase behavior. Cholesterol, constituting up to 40% of mammalian lipid membranes^{28,29}, induces the liquid-ordered (Lo) phase. In this phase, lipids maintain high lateral mobility while their hydrocarbon chains exhibit significant conformational order²⁸. This results in enhanced membrane stability, reduced permeability, increased thickness, and retained fluidity essential for information exchange^{28,30}. Other natural sterols, like ergosterol in yeast and stigmasterol in plants, exhibit analogous properties³¹. Similarly, bacteria employ hopanoids, such as diplopterol, to perform comparable functions to sterols^{32,33}.

The phase behavior of ternary mixtures containing cholesterol (or other sterols) can be characterized using Gibbs phase diagrams (Figure 1b). In the regions of phase separation in these triangular diagrams, the lipid compositions of coexisting phases are defined by tie lines (straight lines connecting the compositions of phases that coexist in equilibrium) and their relative proportions can be determined using the lever rule, which relates the overall composition to the fractional amounts of each phase along a given tie line (see ref. ³⁴ for a detailed summary).

Although biological membranes are far more complex than simple lipid mixtures, incorporating proteins and many other components, phase diagrams remain invaluable tools. They help us understand fundamental lipid interactions and how phase behavior can be modified by various parameters. Importantly, many properties of natural membranes can be reproduced in lipid-only model systems³⁵, highlighting their importance for dissecting biological processes.

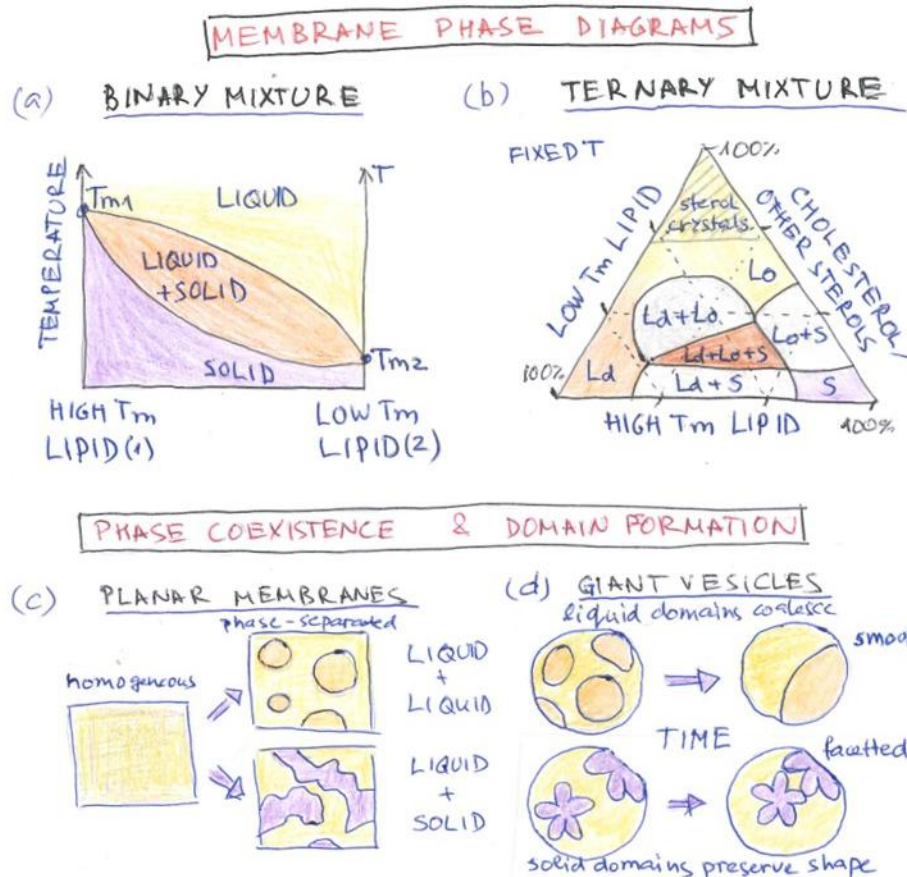


Figure 1. Lipid phase separation and phase diagrams. Examples of phase diagrams (a, b) and domains appearance as a result of phase separation (c, d). (a) Phase diagram for a binary lipid mixture as a function of temperature and lipid composition. Mixtures of lipids with a high melting temperature (T_{m1}) and a low melting temperature (T_{m2}) can exhibit coexistence of liquid crystalline ($L\alpha$) and solid (gel, S_0) phases. (b) Ternary phase diagram (also referred to as **Gibbs triangle [G]**) for a three-component lipid mixture (e.g. high T_m lipid, low T_m lipid and cholesterol) where vertices represent the three components, with increasing concentration read counter-clockwise. At temperatures between T_{m1} and T_{m2} , these membranes can exhibit coexistence of liquid-ordered (L_o), liquid-disordered (L_d) and/or solid (S_0) phases. (c) Phase separation in planar membranes is visualized as round domains during liquid (L_d and L_o) coexistence or as faceted or irregular domains during the coexistence of solid–liquid crystalline phases. (d) In giant vesicles, liquid domains are typically round and coalesce, reflecting their fluidity, whereas solid domains exhibit edges, preserve their shape and do not merge, indicating their rigidity.

Box 1 | Membrane lipid phases and domain nucleation and growth

Lipid bilayers exhibit distinct thermotropic phases whose characteristics depend on temperature and lipid composition. Here we discuss the most common examples:

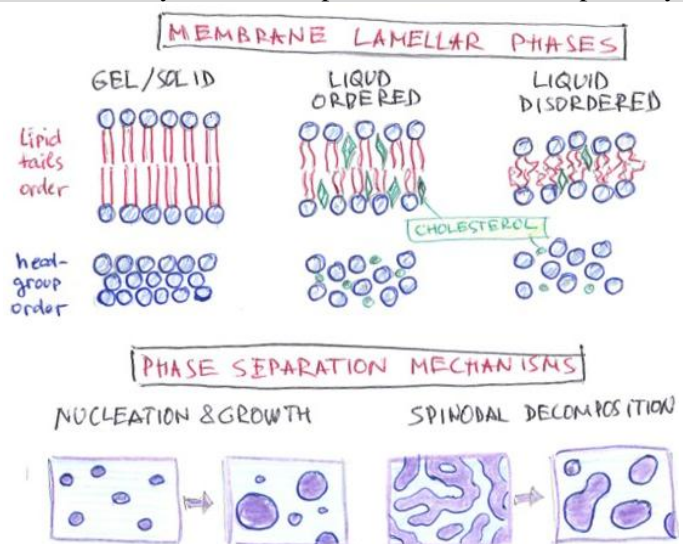
- **Gel or solid (S_0) phase**, which is observed below a lipid's main phase transition temperature (T_m) and is characterized by high lipid chain order, very slow translational movement, and ordered headgroups.
- **Liquid-crystalline ($L\alpha$) phase**: This is observed above T_m and features disordered hydrocarbon chains and polar headgroups, with high translational diffusion (that is, high Brownian motion).
- **A liquid-ordered (L_o) phase** forms in the presence of cholesterol or other sterols and combines high acyl chain order (tight packing) with high lateral fluidity (rapid lipid diffusion), conferring membranes with reduced permeability and increased thickness. Cholesterol fluidizes rigid, high- T_m lipid membranes (e.g., those enriched in dipalmitoylphosphatidylcholine (DPPC)) by disrupting gel-phase packing and reducing headgroup order³⁶. Conversely, in highly fluid, low- T_m membranes (e.g., 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)) cholesterol increases chain order while maintaining fluidity, in which case the state is referred to as **liquid-disordered (L_d) phase**. The L_d phase is less ordered than the L_o one and is

characterized by loosely packed hydrocarbon chains with significant conformational flexibility and more rapid lateral diffusion of lipids.

Different lipid phases can coexist (e.g., S_o – L_α or L_o – L_d), and three-phase coexistence (S_o – L_o – L_d) is also possible. The emergence of a new phase within a homogeneous membrane can occur through different mechanisms:

- **Nucleation** occurs when the system is quenched into a metastable region. Small clusters or domains of the minority phase initially form, requiring the system to overcome an energy barrier to initiate phase separation. Once stable, these nuclei grow by recruiting more molecules, merging with other clusters or domains (e.g. via collision and coalescence), or undergoing Ostwald ripening (where larger, more stable domains grow at the expense of smaller ones)^{37,38}.
- **Spinodal decomposition** occurs when the system is quenched into an unstable region, where even infinitesimal compositional fluctuations spontaneously grow without any thermodynamic energy barrier³⁹. This diffusion-controlled process typically produces fine, interconnected domains with a characteristic length scale that depends on the composition along the tie line. Over time, these domains coarsen and can undergo Ostwald ripening³⁷.
- **Critical fluctuations:** Near a **critical point [G]**, the nucleation barrier for phase separation vanishes, and the **line tension [G]** approaches zero. The system exhibits large-scale, transient compositional fluctuations known as "critical fluctuations", which continuously form and dissolve without giving rise to stable domains. These fluctuations can significantly affect membrane organization and dynamics, independent of the kinetic pathway by which phase separation occurs.

In membranes with coexisting fluid–fluid (L_o – L_d) phases, phase separation can proceed by either mechanism due to the high mobility of lipids. In contrast, fluid–gel systems show slower domain evolution, rarely exhibit spinodal patterns, and often display anisotropic domain shapes due to the rigid, ordered nature of the gel phase. Although both nucleation and spinodal decomposition lead to domain coarsening, their initial genesis fundamentally differs through the presence or absence of an energy barrier, respectively³⁸. These general physical principles are thought to underpin “raft” formation in the plasma membrane, although cellular environments introduce complexities such as obstructed mobility and continuous changes in local conditions³⁸.



Resolving membrane domains and their composition, dynamics and stability

Cell membranes exhibit phase separation, forming dynamic domains ranging from nanometers to micrometers. A classic example in model systems is the coexistence of L_o domains (enriched in cholesterol and saturated lipids) with a continuous liquid-disordered (L_d) phase (rich in unsaturated lipids), see Box 1. In natural cell membranes, these domains, often referred to as rafts²¹, serve as specialized platforms. Their specific lipid and protein composition, coupled with their dynamics and stability, dictates their diverse roles in cellular processes such as signal transduction, protein sorting, and pathogen infection^{20,22}, see ref. ²¹ for a comprehensive Review.

Membrane domains can arise through mechanisms like nucleation and spinodal decomposition (Box 1). Additionally, nanodomains can emerge from critical fluctuations. Evidence suggests that the plasma membrane operates near a phase transition, making it susceptible to "critical phenomena" where nanodomains can form from the fragmentation of larger domains, persisting at milliseconds below physiological temperatures²⁰. Studies in model systems, **supported lipid bilayers [G]**, nanoscopic and giant vesicles, have been instrumental in characterizing these phenomena^{21,40,41}. Distinguishing between liquid–liquid and liquid–solid phase separation is often evident from domain morphology that can be assessed with various microscopy approaches: liquid domains tend to be round and dynamic, whereas solid domains do not coalesce^{27,34} (Figure 1c, 1d). In some cases, known as **modulated phases [G]**, lipid mixtures can exhibit periodic structural patterns, such as stripes or dots. These patterns result from a complex interplay of lipid packing, **membrane spontaneous curvature [G]**, elasticity and tension, and electrostatic interactions⁴²⁻⁴⁴.

It is important to note that observations of phase behavior in model systems can be influenced by factors such as the presence of a supporting substrate, system geometry, and membrane tension. These variables can be precisely

controlled or systematically varied in giant unilamellar vesicles (GUVs)^{34,45} [G], making them powerful tools for studying intrinsic membrane properties despite the caveat of a minimalistic composition. The relevance of phase separation in biological membranes is further supported by experiments on giant plasma membrane vesicles (GPMVs). These blebs retain the native lipid and protein diversity of cellular plasma membranes and exhibit phase separation into microdomains with specific protein and lipid compositions^{46,47}. This result indicates a tendency for lateral segregation of components in living membranes²⁰.

The shape, amount, and distribution of domains are influenced by domain line tension, the one-dimensional equivalent of surface tension⁴⁸, which drives the system to minimize boundary length and favors circular domains. Line tension itself depends on how far the membrane is from a critical point, decreasing as criticality is approached and vanishing at the critical point. However, line tension alone does not determine domain size: in a simple system with finite line tension, the equilibrium state corresponds to a single large domain. The presence of multiple smaller domains typically reflects kinetic trapping, additional repulsive interactions, or observation before full equilibration. Although some lipids with acyl chain mismatch were hypothesized to act as “lineactants”, experiments in model systems suggest that no specific structural features are required; rather, molecules that can be accommodated within membrane defects may have a role^{49,50}. Certain proteins or DNA can decrease line tension, arresting domain coalescence^{48,51}. Ultimately, the number and distribution of domains in a membrane arise from a complex interplay between proximity to a critical point, line tension, supersaturation, kinetic factors and membrane dynamics³⁸.

Phase separation in biological membranes

Biological membranes are exceptionally dynamic and heterogeneous, composed of over a thousand distinct lipid species²⁹ and up to 50% protein by mass. This inherent complexity strongly suggests a predisposition for lateral component segregation and membrane heterogeneity. However, direct observation of stable, micrometric domains in vivo is rare^{20,52}. Instead, experiments primarily based on super-resolution and fluorescence correlation spectroscopy, revealed that domains in biological membranes are predominantly nanometric and highly transient⁵³⁻⁵⁶. The only exception so far is the vacuole membrane of yeast, where microscopic phase separation has been directly observed^{57,58}. The size and dynamics of membrane domains in cells are modulated by interactions with the underlying cytoskeleton and diffusion-restricting “fences”^{38,59,60}. These structures arise from transmembrane proteins anchored to the cytoskeleton, forming a mesh that hinders lipid and domain diffusion, restricts domain coalescence, and increases viscosity^{38,59,60}. This, coupled with the cellular fluctuations, favors smaller, highly dynamic domains³⁸. This inherent dynamism (constant forming, coalescence, and dissolving on sub-second to second timescales) is critical for their biological function, enabling rapid reorganization and signaling pathway activation⁶¹ (see below).

Condensate formation in 3D bulk

Macromolecules like proteins, peptides and nucleic acids can undergo liquid–liquid phase separation, forming dense and dilute phases above a **saturation concentration [G]**, modulated by environmental cues (e.g., temperature, pH, ionic strength). Here, we explore the diverse mechanisms of protein and polymer phase separation in bulk. We will highlight how in vitro reconstituted systems provide molecular insights into condensate formation and review approaches for assessing their material properties both in vitro and within cells.

Types of phase separation

Liquid–liquid phase separation (LLPS) is a fundamental physicochemical process where an initially homogeneous macromolecular solution separates into two coexisting aqueous phases: a concentrated dense phase and a less concentrated dilute phase (Figure 2a). However, for biomolecular condensates, the term “LLPS” can be an oversimplification. Although classical LLPS describes the coexistence of two liquid phases, many biological condensates exhibit a wider range of material behaviors and can be more gel-like or networked rather than behaving as simple liquids. As highlighted in recent discussions on terminology, many biomolecular condensates do not represent two simple liquids and may fall outside the classical LLPS thermodynamic framework defined by the Flory-Huggins theory⁶². These condensates or membraneless organelles⁶³, exemplified by the nucleolus, stress granules, presynaptic densities, and P-bodies, form through multivalent, often weak and transient, interactions between macromolecules, typically involving intrinsically disordered regions (IDRs). These interactions are frequently described by a “stickers-and-spacers” sequence organization model, where specific residues or motifs (“stickers”) drive associations separated by flexible linkers (“spacers”)⁶⁴, leading to condensates with a wide range of physical properties. In the following, we use “LLPS” as a convenient umbrella term to describe demixing driven by such multivalent interactions, while acknowledging that biological condensates can span a wide spectrum of material states from low-viscosity liquids to viscoelastic gels and more solid-like states.

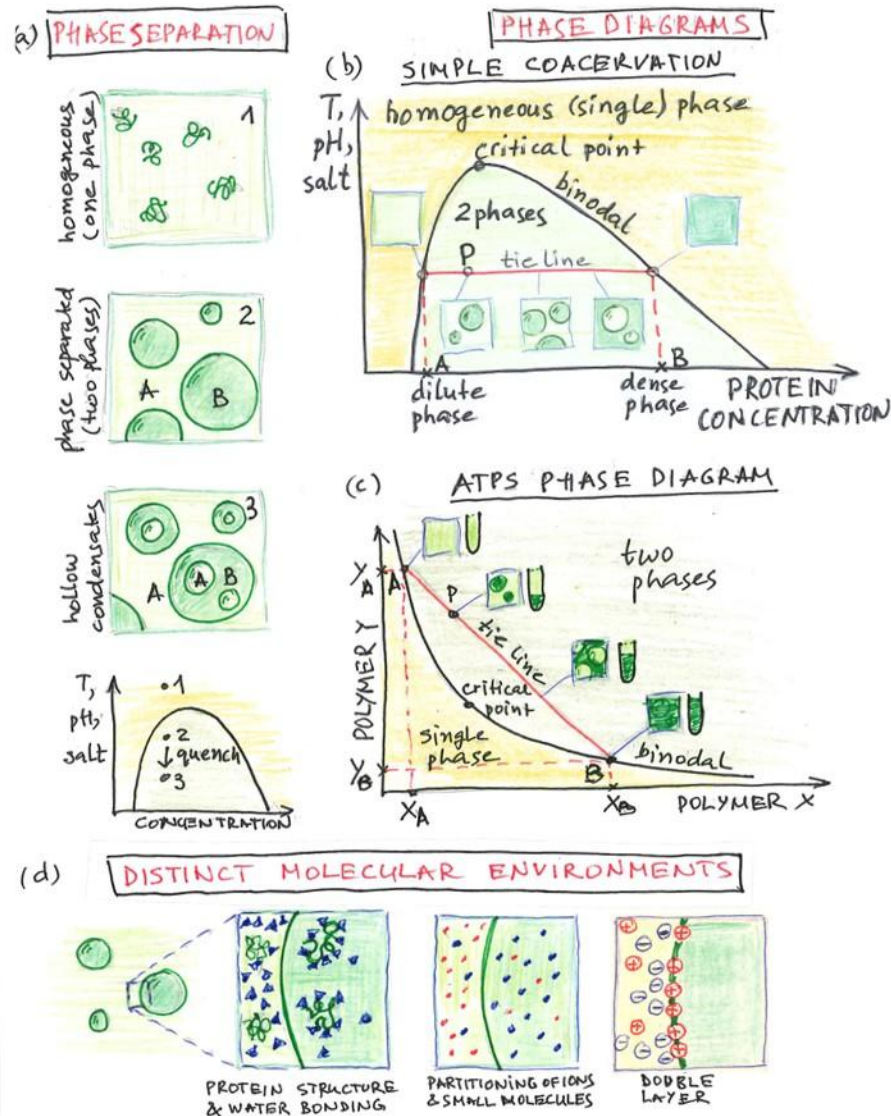


Figure 2. Macromolecule phase separation and basic condensate properties. (a) Macromolecules ① can undergo liquid–liquid phase separation ② forming a concentrated dense phase (condensates, droplets) in equilibrium with a less concentrated (dilute, depleted) phase. Quenching of preformed condensates by shifting conditions (e.g., salt, temperature, pH) can induce internal reorganization, leading to multiphase structures such as hollow condensates ③. The diagram on the right shows an example pathway in the phase diagram reflecting the states ①– ③ sketched on the right. (b) A generic protein phase diagram showing macromolecule concentration against a tunable parameter (e.g., temperature, pH, ionic strength). (c) A phase diagram of aqueous two-phase systems (ATPS) illustrates phase separation occurring isothermally based on the concentrations of the two macromolecules or polymers. In both (b) and (c), the binodal delineates the boundary of the two-phase coexistence region; outside of this boundary, the system is homogeneous (single phase). At the critical point, the two phases become indistinguishable. For an overall system composition (e.g., P), a tie line connects the compositions (A, B) of the two coexisting phases in equilibrium. The lever rule then allows calculating the relative amounts (e.g., volume fractions as illustrated) of these two coexisting phases. (d) Examples of the distinct molecular environments within the dense and dilute phases, including variations in macromolecular structure and hydration by water molecules (triangles), an asymmetric partitioning of ions and various small molecules (indicated by different colored dots), and the presence of an interfacial double layer that generates an electric field capable of promoting chemical reactions.

Similar to two-dimensional (2D) phase separation in lipid mixtures, phase diagrams can be constructed for three-dimensional (3D, bulk) macromolecular phase separation (Figure 2b). These diagrams map condensate formation based on macromolecule concentration and environmental parameters like temperature, salinity, or pH. Inside the

boundary defined by the **binodal [G]** curve, dense (e.g. protein-rich) and dilute (or depleted, protein-poor) phases coexist; outside, the system remains homogeneous.

Phase separation can be broadly categorized as associative and segregative⁶⁵:

- **Associative** phase separation is driven by attractive interactions between components. An example of associative LLPS is coacervation — a phase separation process in which polymers or biomolecules spontaneously condense into dense, liquid-like droplets, whereas the surrounding solution remains dilute. This can be “simple” (or self-) coacervation when a single component interacts with itself, or “complex” coacervation involving oppositely charged components⁶⁶. Many proteins and polymers self-coacervate, and oppositely-charged polypeptides commonly form complex coacervates.
- **Segregative** phase separation is driven by repulsive interactions between the components which demix into distinct phases. A well-studied example is the **aqueous two-phase system (ATPS) [G]**, typically formed by mixtures of polymers like polyethylene glycol (PEG) and dextran. These systems phase-separate based on concentration (Figure 2c). Reported as early as the late 19th century with gelatin and starch⁶⁷, ATPS predates LLPS conceptually and has been extensively used in biochemistry and chemical engineering for the separation and purification of biomolecules, organelles, and cells based on differential solubility⁶⁸.

Condensates can present a multilayered structure, with internal compartments of different compositions⁶⁴⁻⁶⁹. A clear example of such architecture is the nucleolus that is constituted by distinct liquid-like subcompartments, each of which is involved in different steps of ribosome production⁶⁹. The mechanism behind the formation of these structures is proposed to result from dynamically arrested phase separation⁶⁶: as an in vitro example, when preformed isotropic spherical condensates are exposed to a sudden change in external conditions (e.g. salinity, compositional changes, pH), they can undergo internal reorganization and multiphase structuring, such as forming shell-like hollow droplets⁶⁹⁻⁷² (Figure 2a).

For an in-depth introduction to the thermodynamics of liquid–liquid phase separation and current models describing intracellular condensates, see references^{62,64,65}.

Condensate properties

Like membranes, biomolecular condensates are supramolecular structures exhibiting emergent properties at the **mesoscale [G]** — characteristics that cannot be necessarily deduced from their individual molecular components. Key emergent properties of condensates include viscosity, surface tension, and micropolarity (Box 2 summarizes experimental techniques for measuring these).

Viscosity is a measure of a fluid’s resistance to flow (honey is ~10000 times more viscous than water), and can be orders of magnitude higher in the dense phase of condensates (up to 300 times that of bulk water), reflecting macromolecular concentrations thousands of times greater than in the dilute phase⁷³. In fluid condensates, molecules can nevertheless remain highly dynamic, bound by short-lived interactions that exchange on pico- to nanosecond timescales⁷⁴, but as we will discuss below, some condensates in cells can behave as highly viscous or even arrested assemblies with markedly reduced molecular mobility.

Surface tension, which quantifies the energetic cost of increasing a liquid’s interfacial area, is a critical parameter governing capillary phenomena within cells⁷⁵ and decreases as a system approaches a critical point. Analogous to how line tension promotes domain merging in membranes, surface tension shapes condensates into spheres, drives their coalescence, and regulates wetting of membranes, the cytoskeleton, and DNA⁷⁵. Alongside electrostatic properties, surface tension is crucial for the organization of multiphase condensates like the nucleolus^{76,77}.

Micropolarity (or hydrophobicity), often quantified by the dielectric constant (ϵ), is an important parameter closely linked to viscosity and surface tension^{11,76-78}. It dictates condensate interactions with surrounding organelles and the partitioning of small molecules. Micropolarity and water content measurements can thus provide a unique “fingerprint” of a condensate-specific biochemical environment^{11,79,80}.

Box 2 | Methods to study biomolecular condensate properties

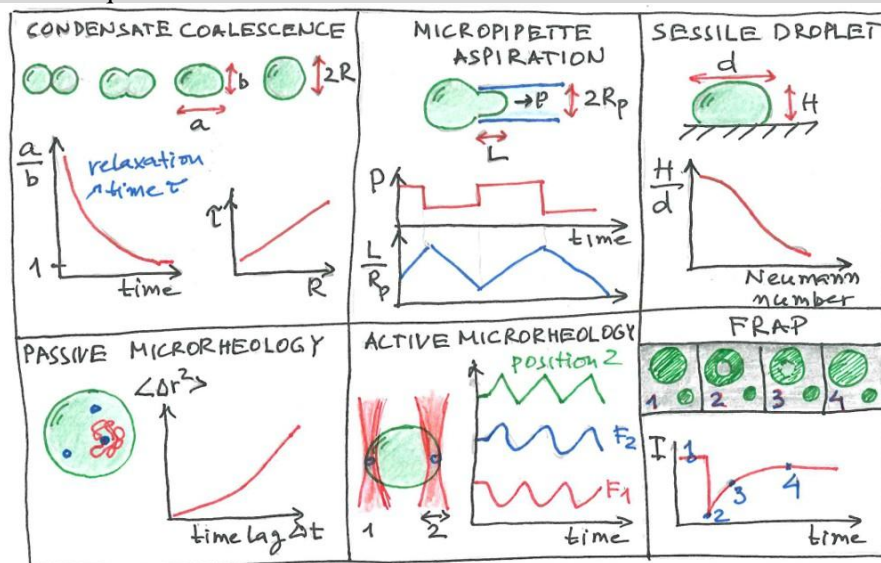
Characterizing the physicochemical properties of biomolecular condensates is crucial to understand their function. Various microscopy-based and spectroscopic techniques enable the measurement of properties like surface tension, viscosity, and micropolarity.

Viscosity and surface tension:

- a) *Condensate coalescence*: The rate at which condensates coalesce, quantified by their inverse capillary velocity, provides a combined measure of viscosity and surface tension. This requires tracking the time-dependent change in the aspect ratio (a/b) of merging droplets, ideally of similar sizes, using optical

microscopy. The characteristic time τ scales linearly with the final condensate radius R , and the slope of this relationship gives the inverse capillary velocity.

- *b) Micropipette aspiration:* This direct method allows precise quantification of both surface tension and viscosity of condensates, applicable to both in vitro systems⁸¹ and living cells^{82,83}. The condensate is aspirated into a micropipette of known radius (R_p), and variable suction pressure P is applied while monitoring the time-dependent response of the aspirated length L .
- *c) Sessile droplet method:* This approach measures surface tension by analyzing the equilibrium shape of a condensate droplet sitting on a surface and its deformation by gravity^{84,85}. The vertical contour of the sessile droplet is extracted from microscopy images and fit to the capillary shape predicted by the **Young–Laplace equation [G]**, enabling determination of the surface tension. For accuracy, droplets with Neumann number greater than 0.3 must be used, a threshold at which gravitational deformation becomes sufficiently large relative to surface tension for a reliable mathematical fit. By quantifying the droplet shape and the density difference between the coexisting phases, the interfacial tension can be determined.
- *d and e) Microrheology* utilizes micron-sized beads embedded within condensates to determine their viscosity and viscoelasticity. *Passive microrheology* quantifies Brownian motion, whereas *active microrheology* employs optical tweezers to apply forces and measure bead response^{86,87}. In passive microrheology, the diffusion coefficient and viscosity are determined by measuring the mean squared displacement ($\langle \Delta r^2 \rangle$) of a bead, which quantifies its thermally driven random motion over a lagtime (Δt). In active microrheology, one bead is deliberately displaced while the forces exerted by the optical traps on both beads (F_1 and F_2) are measured, providing direct information about complex rheological parameters. Optical tweezers can also probe thermal fluctuations of trapped particles within condensates⁸⁸. These rheological techniques are vital for characterizing condensate viscoelasticity in response to buffer changes⁸⁹, aging⁸⁷, and polypeptide amino acid and nucleotide sequence⁹⁰.



Molecular dynamics and exchange:

- *Fluorescence recovery after photobleaching (FRAP (panel f)):* A widely used technique to determine the diffusivity of condensate components and their exchange kinetics with the surrounding dilute phase. In this technique, the fluorescence intensity (I) at the bleached spot is monitored over time, enabling determination of the characteristic recovery half-time, the diffusion coefficient and the immobile fraction of the macromolecules inside the condensate.
- *Fluorescence correlation spectroscopy (FCS):* This approach offers a more reliable quantification of molecular diffusion within condensates^{91,92}. Advanced variations like line raster image correlation spectroscopy (RICS)⁹³ are particularly useful for characterizing dynamic properties of condensates in cells⁹⁴.

Local environment:

- *Micropolarity or hydrophobicity:* Microscopy methods like hyperspectral imaging and fluorescence lifetime imaging microscopy (FLIM), which employ environment-sensitive dyes, quantify changes in micropolarity or the dielectric constant, reflecting the local hydrophobic environment^{11-13,77,79,95}.

- *Vibrational spectroscopy*: Complementary methods such as Raman and Fourier-transform infrared (FTIR) spectroscopy provide insights into macromolecular conformational changes^{11,96} and the content and coordination of water within condensates^{11,79,80}.

In vitro reconstitution systems are invaluable for dissecting the complexities of cellular condensates (often composed of hundreds of protein or proteins and genetic material⁹⁷), enabling researchers to reduce component numbers and precisely manipulate physicochemical parameters. Much of our understanding of condensate behavior stems from combining in vitro, in silico, and cell experiments. For example, in vitro studies have revealed the “molecular grammar” of condensate formation and their programmable viscoelasticity^{90,98,99}, showing how specific amino acid sequences and regions drive phase separation⁹⁸, and how subtle variations in peptide and RNA sequences can yield condensates with very different material properties^{90,100,101}. In this sense, condensates are best viewed as metastable viscoelastic fluids spanning a wide range of viscoelastic properties, from liquid-like to highly viscous or near-arrested states^{87,102,103}. Environmental factors profoundly impact condensate properties. Studies on glycinin condensates, for example, have demonstrated that salinity changes can induce protein secondary structure reorganization, directly affecting water dynamics and altering condensate material properties¹¹. Correlations between collective water behavior and micropolarity measured by probes like 2-acetyl-6-(dimethylamino)naphthalene (ACDAN)¹¹ emphasize the crucial role of electrostatics in phase separation. Indeed, changes in buffer composition can alter the material and electric properties of condensates, highlighting the relevance of ions and the surrounding medium^{11,78,89,104-106}. Figure 2d illustrates how phase separation leads to distinct differences in macromolecular structure, water bonding, ion and small molecule distribution, and the formation of an interfacial ionic double layer^{105,107,108}. These asymmetries have consequences for intermolecular interactions and molecular partitioning¹⁰⁵, and the interfacial potential can even drive chemical reactions^{109,110}.

Inside the cell, condensation generates unique collective properties that expand the functional repertoire of cells. For example, condensates can locally concentrate specific proteins, buffering cellular noise while maintaining signaling robustness despite fluctuations in gene and protein expression¹¹¹, act as reaction crucibles¹ and form dense networks that act as a semi-permeable barriers, enabling biochemical compartmentalization¹¹². More generally, their tunable material properties and dynamic assembly allow cells to organize reactions spatially and temporally, selectively include or exclude components, and rapidly respond to environmental or signaling cues.

It is important to highlight that whereas most in vitro systems are evaluated under thermodynamic equilibrium, condensate formation, size regulation, and spatial organization in vivo are often governed by active non-equilibrium processes. Under equilibrium, droplet size is defined by minimization of interfacial tension, and growth proceeds through coalescence and **Ostwald ripening** [G]. However, in cells this behavior can be altered by active chemical reactions, cytoskeletal interactions and mechanical constraints⁶⁵. Some in vitro systems have begun to recapitulate such non-equilibrium regulation. For example, fueled coacervates, in which phase separation is coupled to a chemical reaction cycle, demonstrate how energy dissipation can suppress coarsening and thereby control droplets size and distribution^{103,113}. Enzyme-containing droplets can also migrate directionally by generating local chemical gradients¹¹⁴. These active model systems allow selective and controlled manipulation of enzymatic activities, protein concentrations, and other physicochemical parameters, thus providing key insights into how kinetics and out-of-equilibrium processes modulate phase separation and condensate properties.

Finally, **condensate aging** [G] is a process often linked to neurodegenerative pathologies, and can mediate the nucleation of amyloid fibers^{3,115}. During aging, key properties such as surface tension, viscosity, and electrochemical potential change^{63,87,95}, highlighting their key role in condensate function and cellular fitness⁶³.

Coupling 2D and 3D phase separation

Coupling 2D and 3D phase separation is important in cellular organization, as membranes actively modulate biomolecular condensate nucleation and assembly. This section explores two key modes of interaction: receptor-mediated condensation and the direct interaction of 3D condensates with membranes, even without specific molecular anchors. We will review capillary-driven phenomena during condensate wetting of membranes, alongside experimental and theoretical approaches quantifying these interactions. Finally, we discuss how 2D membrane phase separation can be coupled to 3D macromolecular phase separation in the cytoplasm.

Protein phase separation at the membrane surface

Membranes serve as active platforms that can drastically reduce the concentration threshold for protein phase separation in bulk, promoting the assembly of 2D condensates at their surface. This phenomenon, termed prewetting

or surface condensation, occurs when proteins bind to the membrane via specific tethers or lipid interactions^{116,117}. Although direct comparison of 2D (molecules/ μm^2) and 3D (molecules/ μm^3) concentrations is challenging due to differing units, data strongly suggest that localizing phase-separating components to membranes allows cells to leverage lower critical concentrations to drive condensate formation and function at specific membrane locations¹¹⁸. Prewetting fundamentally relies on the reduction in dimensionality from 3D bulk to a 2D surface^{119,120}, which increases the effective local concentration of membrane-binding proteins (Figure 3a). These 2D condensates, appearing as domains within the membrane, exhibit liquid-like properties such as diffusion and coalescence^{116,117,121,122}.

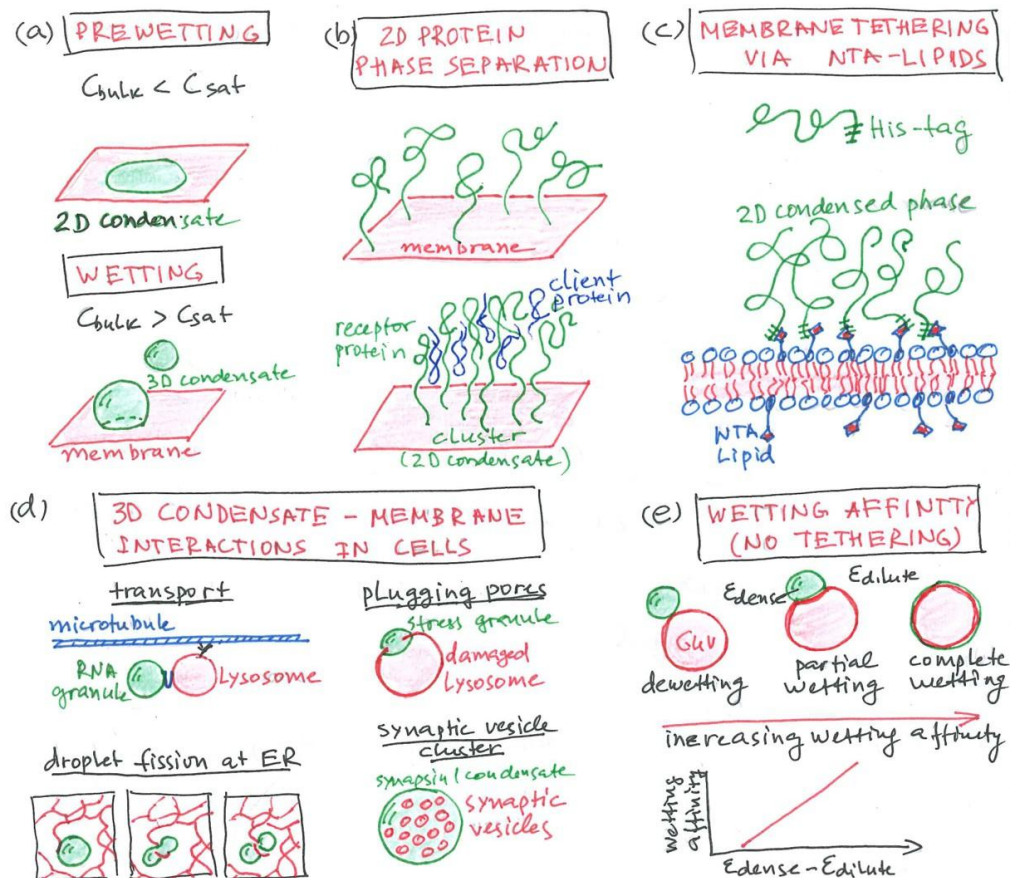


Figure 3. 2D and 3D condensate formation and reconstitution in vitro. (a) Prewetting and wetting of membranes by biomolecular condensates. Prewetting describes the formation of 2D condensates at the membrane surface, occurring below the bulk saturation concentration. Wetting refers to the interaction of pre-formed 3D condensates from the bulk with membranes. (b) Receptor clustering in biological membranes drives 2D condensate formation. (c) 2D condensates can be reconstituted in vitro using NTA lipids, which bind His-tagged proteins through nickel coordination. (d) Cellular examples of 3D condensate–membrane interactions, where membraneless organelles (3D condensates) interact with membrane-bound organelles: RNA granules associate with lysosomes (mediated by Annexin A11) for microtubule-based transport, stress granules plug and stabilize damaged endolysosomes, the ER membrane mediates fission of ribonucleoprotein (RNP) granules, and synaptic vesicles are clustered by synapsin-1 condensates. (e) 3D condensate–membrane interactions can be reconstituted in vitro using GUVs and protein condensates or peptide coacervates. Condensate–membrane affinity can be precisely tuned by modulating buffer salinity, membrane charge, or cholesterol content. A direct correlation between the dielectric constant of the dense and dilute condensate phases and wetting affinity highlights the importance of both phases in determining these interactions.

Receptor-mediated condensation is a common mechanism for 2D condensate formation at membranes (Figure 3b-c). Here, membrane-associated proteins on the cytoplasmic side of the membrane, including transmembrane or peripheral proteins that act as receptors or tethers (sometimes referred to as “scaffolds”), drive condensate formation and subsequently recruit and concentrate “client” molecules, which can, in turn, influence the phase behavior and diverse

functions of the condensates. In vitro reconstitution studies have been instrumental in dissecting these processes. A popular method to tether proteins to model membranes involves Ni-nitrilotriacetic acid (NTA) lipids, which efficiently chelate His-tagged proteins^{17,19,122,123} (Figure 3c). For example, the membrane-bound phosphorylated Nephryn protein forms condensates with the SH2/SH3 adaptor protein Nck and neural Wiskott-Aldrich syndrome protein (N-WASP, also known as WASL) when tethered to supported bilayers via NTA lipids¹⁹. One of the most studied 2D condensates involved in signaling is that formed by the T-cell receptor linker for activation of T cells (LAT). Phosphorylated LAT (pLAT) interacts with growth factor receptor-bound protein 2 (Grb2) and son of sevenless (Sos), driving micron-sized condensate formation in supported bilayers^{17,18}. Beyond NTA lipids, other approaches utilize glycosylphosphatidylinositol (GPI)-anchored proteins or recruitment via specific interactions with phosphatidylinositol phosphate (PIP) lipids²³.

Prewetting effectively explains how proteins, which might only phase-separate at high concentrations in vitro, can form condensates in vivo at much lower endogenous levels due to favorable interactions with membrane components and additional client molecules.

3D condensates in contact with membranes

Membranes, being ubiquitous and forming the envelopes of many organelles, frequently interact with membraneless organelles during various cellular processes. Examples include RNA granule transport¹²⁴, lysosome patching by stress granules¹⁰, RNP granules fission at the ER contact sites¹²⁵, and the formation of synaptic vesicle clusters⁸ (Figure 3d). Whereas specific tethers (e.g., Annexin A11 for RNA granules) can mediate these interactions¹²⁴, membrane wetting by condensates can also occur in the absence of molecular anchors. This non-specific interaction is regulated by membrane surface charge, salinity, lipid composition, and the degree of lipid packing and cholesterol content, which finely tune condensate–membrane affinity^{4,69,126,127}.

Wetting transitions describe the degree of interaction between a 3D condensate and a membrane, ranging from dewetting (no interaction), to partial wetting (condensate and membrane mutually reshape), to complete wetting (maximum interaction, condensate spreads over the membrane)^{69,128}. A recent non-peer reviewed article suggests that wetting affinity depends on the **permittivity [G]** contrast between the dense and dilute phases of condensates⁷⁹, as shown in Figure 3e. The first descriptions of wetting transitions involved ATPS encapsulated in GUVs, where polymer concentration modulated condensate–membrane affinity¹²⁸. Later, similar transitions were observed for protein and polypeptide condensates, where buffer salinity was found to have a key role^{69,127}. Even in intrinsically dewetting systems, the inclusion of lipid tethers (e.g., NTA lipids^{122,129}, cholesterol-tagged RNA¹³⁰, or charged lipids^{69,127,130}) can promote condensate wetting.

The condensate–membrane affinity can be quantified by measuring the contact angles formed at the contact line between the droplet and the membrane^{69,131}. Figure 4a shows that the droplet divides the membrane into two segments, the *ic* and the *ie* segments defining three contact angles: θ_i , θ_e , and θ_c . These angles are not material properties, and depend on the system geometry (vesicle and condensate size and available membrane area for deformation). Indeed, the membrane kink that they define at the three-phase contact line does not persist to nanometer scale as the membrane is smoothly curved^{131,132}, and the membrane wetting is defined by a different — intrinsic — contact angle, which is a material property and can be deduced as follows¹³³. The three microscopic angles are related to the interfacial tensions of the two membrane segments (Σ_{ie}^m and Σ_{ic}^m) and the droplet surface tension (Σ_{ce}), which are in equilibrium and form the sides of a triangle (see Figure 4a). This force balance implies the geometric relationships $\frac{\Sigma_{ie}^m}{\Sigma_{ce}} = \frac{\sin \theta_c}{\sin \theta_i}$ and $\frac{\Sigma_{ic}^m}{\Sigma_{ce}} = \frac{\sin \theta_e}{\sin \theta_i}$. The two membrane tensions can be represented as a superposition of the lateral stress in the membrane Σ and the adhesion free energies per unit area of the condensate (W_{mc}) and of the external depleted phase (W_{me}), relative to the interior solution: $\Sigma_{ic}^m = \Sigma + W_{mc}$ and $\Sigma_{ie}^m = \Sigma + W_{me}$ ¹³³. The affinity contrast between the condensate and the external buffer is then given by $W = W_{mc} - W_{me} \equiv \Sigma_{ic}^m - \Sigma_{ie}^m$. Together with the geometric relationships above, one finds that $W = \cos \theta_e^{in} \Sigma_{ce}$, where $\theta_e^{in} = \arccos\left(\frac{\sin \theta_e - \sin \theta_c}{\sin \theta_i}\right)$ is the intrinsic contact angle^{126,131} — the material property defining the membrane curvature at the three-phase contact zone¹³². Care must be taken in measuring the apparent contact angles with confocal microscopy to ensure the rotational axis of symmetry of the GUV–condensate system is in the image plane for accurate analysis⁶⁹. The intrinsic contact angle provides a straightforward way to quantify condensate–membrane interactions and has been used to analyze factors influencing membrane wetting^{11,69,126,134,135}.

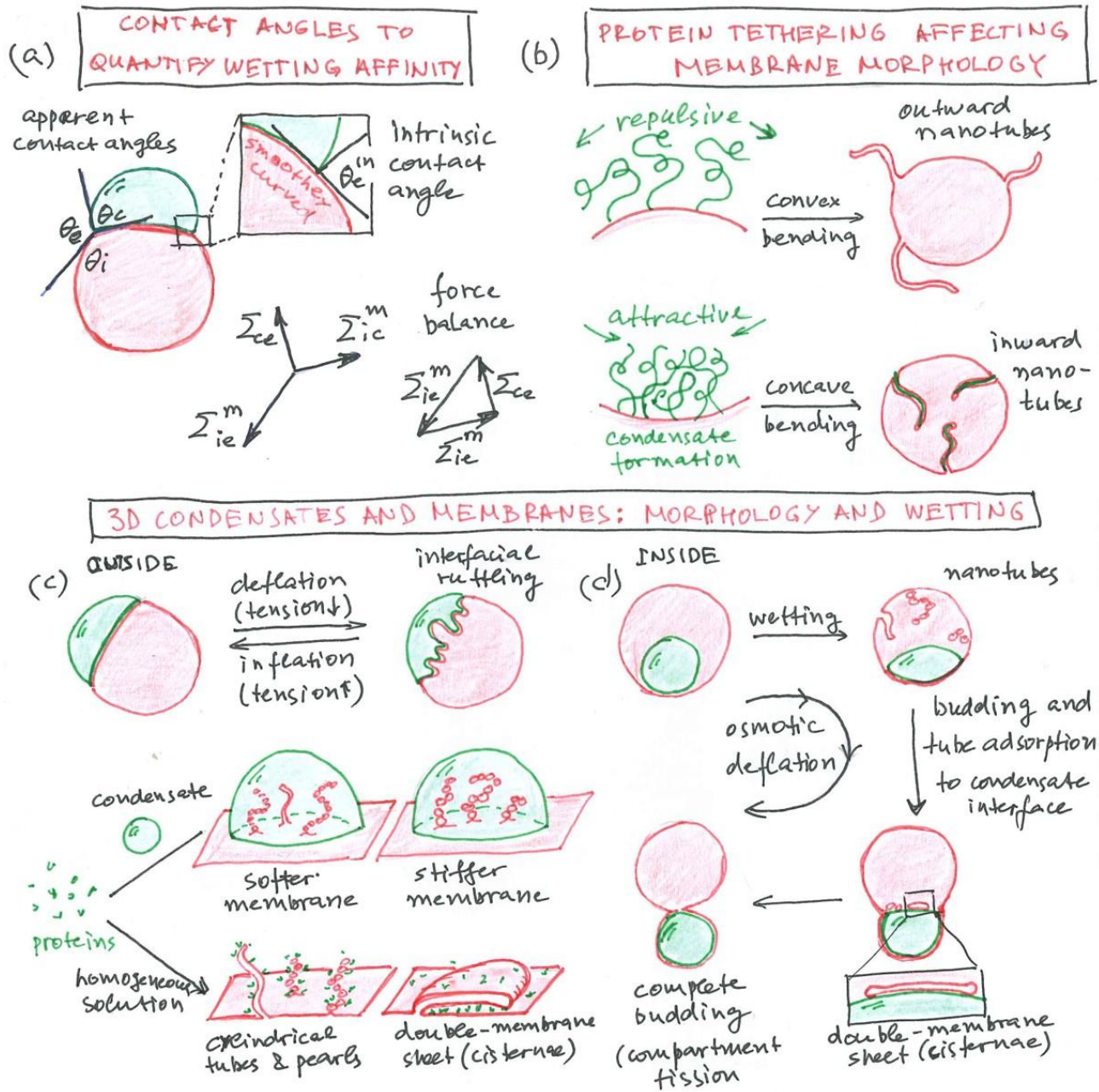


Figure 4. Membrane–condensate mutual remodelling and morphologic transformations. (a) Membrane–condensate interactions can be quantified based on the apparent contact angles, θ_i , θ_e and θ_c , at the three-phase contact line, defined by the droplet surface tension (Σ_{ce}) and the tensions of the two membrane segments ($\Sigma_{ie}^m, \Sigma_{ic}^m$). These tensions are in equilibrium, forming the sides of a triangle. While these apparent angles (observed microscopically) depend on system geometry, the intrinsic contact angle θ_e^{in} (at the nanometer scale) characterizes the true condensate–membrane affinity, representing the smooth membrane curvature at the nanoscale. (b) Tethering intrinsically disordered proteins to membranes drives tubulation. Repulsive protein–protein forces induce convex membrane bending (positive spontaneous curvature) and outward-protruding tubes, whereas attractive forces lead to 2D condensate formation and subsequent inward tubulation. (c) When 3D condensates interact externally with giant vesicles, sufficient excess membrane can lead to interfacial ruffling, forming finger-like structures, which can be retracted by increasing the membrane tension. Wetting can also induce nanotubes that protrude towards the condensates; their thickness depends on membrane rigidity, which is tunable by lipid composition. Beyond condensate-specific effects, general protein–membrane interactions (even from homogeneous protein solutions) can drive nanotube formation and their subsequent transformation into double-membrane sheets adsorbed onto the membrane of origin. (d) Morphological transformations are also induced by internal condensates. In giant unilamellar vesicles

(GUVs) encapsulating internal condensates (e.g., polyethylene glycol-dextran aqueous two-phase systems (ATPS)), wetting transitions, e.g. triggered by polymer concentration via osmotic deflation, drive various morphological transformations, including nanotube formation, budding, asymmetric micro-compartmentation, and the interconversion of nanotubes into double-membrane sheets, stabilized by the condensate interface.

Condensate-induced membrane remodeling and morphological transformations

Upon contact with the membrane, condensates can induce various morphological transformations^{4,135,136}, with important differences depending on whether they form primarily 2D assemblies at the membrane surface or 3D droplets that interact with the membrane as bulk condensates.

Remodeling by 2D condensates When intrinsically disordered proteins (IDPs) bind to the membrane surface, they can induce membrane curvature and nanotube formation. Repulsive protein interactions can lead to convex membrane bending (also referred to as positive spontaneous curvature) and outward-protruding nanotubes, whereas attractive interactions forming 2D condensates can induce concave membrane bending (negative spontaneous curvature) and inward-protruding tubes^{122,137-139} (Figure 4b). Examples include the phase-separating proteins RNA-binding protein FUS, heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNPA2), or intrinsically disordered arginine- and glycine-rich domains (RGG domains) of the transcription factor LAF-1 bound to GUVs via NTA lipids, forming protein-rich patches that induce inward tubulation¹²². Tube thickness depends on membrane rigidity and tension^{140,141}, and is tunable by varying lipid composition, protein concentration, or salinity¹²². Bin, [amphiphysin](#) and Rvs (BAR) domain-containing proteins like endophilin, which are known curvature generators, can form 2D condensates at membranes, promoting vesicle-vesicle adhesion and increasing membrane tension¹⁴².

Remodeling by 3D Condensates When 3D condensates contact vesicles from the outside, mutual remodeling occurs, with the condensate spreading and the membrane curving in response^{69,127} (Figure 3e). If excess membrane is available, the gain in adhesion energy leads to interfacial ruffling, forming stable finger-like structures at the interface, reversible by applying membrane tension^{69,126} (Figure 4c). Nanotubes can also form at the condensate-membrane interface, protruding towards the condensate¹²⁶ (Figure 4c). These tubes, stabilized by spontaneous curvature, can even interconvert into double-membrane sheets (cisternae-like membranes, Figure 4c, 4d)¹²⁶, which are relevant for processes like autophagosome formation¹⁴³ and organelle structures (ER, Golgi)¹⁴⁴⁻¹⁴⁷. The ER for example, is an interconnected network of tubes and rough membrane sheets¹²⁹. Another example is the nuclear envelope, which is composed of two interconnected and continuous membrane sheets¹⁴⁸. Condensate-membrane interactions can also drive spontaneous endocytosis of the condensate, dependent on relative sizes and affinity^{127,134}. Condensates encapsulated within GUVs, as in ATPS, induce various remodeling processes¹³⁵ (Figure 4d). This includes the formation of nanotubes generated by asymmetric polymer adsorption, stabilized by spontaneous curvature^{132,149,150}. These tubes adsorb to the condensate interface and can interconvert into double-membrane sheets, storing excess membrane more efficiently than nanotubes¹⁵¹ (Figure 4d).

Coupling of membrane and protein phase separation

With the increasing observations of membraneless organelles and 2D condensates at membrane surfaces, the long-held view that subcompartmentalization of receptors and functions solely originates from lipid raft formation^{21,152,153} needs to be reevaluated (see next subsection). New findings emphasize the intricate interplay between protein and membrane phase separation, questioning whether earlier observations attributed to lipid rafts in cells may, in fact, have reflected the influence of coupled 2D and 3D condensates on membrane organization. Recent studies demonstrated that protein phase separation can directly induce lipid demixing, for instance, with Src homology 3 (SH3) and precursor membrane (PRM) protein domains anchored to GUVs²⁴. Similarly, the reconstituted T-cell signaling condensates (consisting of LAT, Grb2 and Sos) promote lipid phase separation above the lipid melting temperature²⁵. Here, reciprocal thermodynamic coupling has been established, where condensates regulate the lipid phase state, and in turn, the lipid phase can stabilize protein condensation⁶ (Figure 5a), a synergistic mechanism fundamental for T-cell signal transduction⁶. Proximity to phase transitions in membranes can also promote condensate prewetting. Additionally, the cystic fibrosis transmembrane conductance regulator (CFTR) has been shown to form clusters on cholesterol-enriched membranes¹⁵⁴.

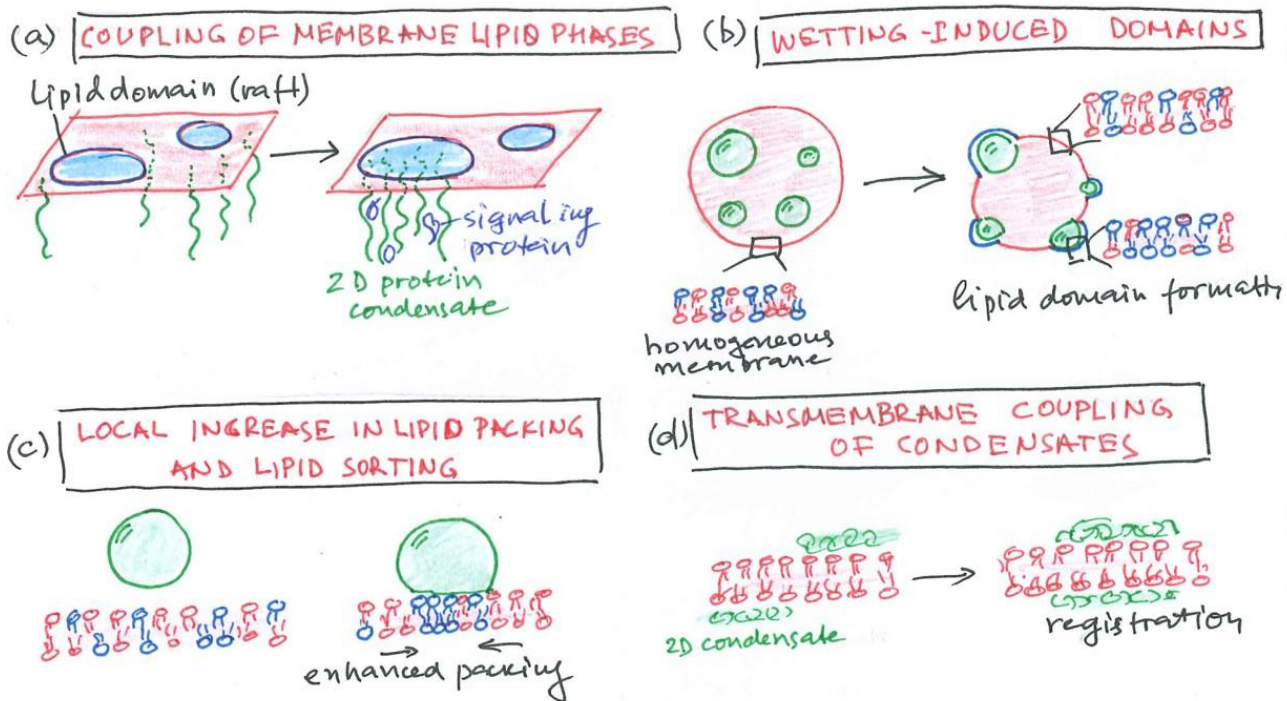


Figure 5. Coupling of membrane and protein phase separation. (a) Reciprocal coupling between lipid domains and protein phase separation. Lipid phase separation can influence protein phase separation at membrane surfaces, and vice versa – protein clustering can induce lipid phase separation. (b) In out-of equilibrium phase separation in giant unilamellar vesicles (GUVs) encapsulating aqueous two-phase systems (ATPS), interaction of dextran-rich droplets with the membrane arrests their coalescence and induces local lipid demixing at the interface. (c) Condensate wetting can increase local lipid packing, dependent on affinity, even in single-component membranes. In multicomponent and charged membranes, condensate wetting further promotes lipid demixing. (d) Condensates initially located on opposite sides of a planar bilayer can undergo transmembrane coupling through the bilayer, forming a single transversal domain.

Coupling between bulk and membrane phase separation is also evident¹⁵⁵. For example, when GUVs encapsulating ATPS undergo osmotic shock, the phase-separated dextran-rich droplets interact with the membrane, arresting their coarsening and promoting local lipid demixing²⁶ (Figure 5b). Furthermore, macromolecular condensates can locally modulate lipid packing, even in the absence of charged lipids or specific interactions¹¹ (Figure 5c). This general mechanism, independent of condensate chemical nature or material properties, depends on affinity and could explain the observed reduction of lipid mobility at membrane–condensate interfaces for both 3D⁶⁹ and 2D²³ condensates.

This mechanism is also thought to facilitate transmembrane coupling of condensates through the bilayer²³ (Figure 5d). Condensates formed on both sides of free-standing planar membranes on transmission electron microscopy (TEM) grids^{38,156-158}, initially nucleated in different regions, have been observed to come into register over time²³, providing strong evidence for inter-leaflet coupling, which is important for cell signal transduction²³.

Molecular dynamics simulations [G] have proven invaluable in predicting and resolving these coupling mechanisms beyond the nanoscale. They have shown local lipid ordering effects in bilayers adjacent to polypeptide-based condensates^{159,160}, and the formation of lipid clusters and sorting of charged lipids at the contact region, dependent on interaction affinity for various systems^{161,162}.

A note of caution on protein-induced lipid phase separation and alternative clustering mechanisms

It is crucial to conceptually distinguish between protein-driven 2D condensates (prewetting), which form a distinct thermodynamic phase, and protein-induced lipid phase separation, which can occur without direct protein–protein condensation, even though these processes exist along a continuum and can appear superficially similar in cells. Protein adsorption, often electrostatically mediated, can locally drive lipid demixing in membranes, causing charged lipids to migrate and form distinct domains, potentially leading to macroscopic lipid phase separation¹⁶³. These phenomena, theoretically predicted^{164,165}, have been experimentally observed: for instance, cytochrome C induces lipid phase separation in otherwise homogeneous membranes¹⁶⁶, endosomal sorting complexes required for transport (ESCRT)-

III proteins (specifically vacuolar-sorting protein 32 (Vps32), in combination with Vps20t) trigger lipid domain formation by binding negatively-charged lipids in GUVs¹⁶⁷, the ganglioside GM1 is clustered by its ligand cholera toxin B leading to the formation of membrane domains¹⁶⁸, and membrane-bound actin networks can induce lipid phase separation¹⁶⁹. Although model membrane studies aid in decoupling these mechanisms, distinguishing 2D protein condensates from protein-enhanced lipid domains in complex cellular environments remains challenging, necessitating careful scrutiny to determine if observed enrichment is primarily driven by protein condensation or protein-induced alterations in lipid phase behavior.

It is also important to emphasize that not all cellular heterogeneities appearing like clusters or puncta arise through phase separation. Local accumulation of macromolecule can also result from alternative processes like binding interactions¹⁷⁰, percolation and gelation¹⁷¹, stoichiometric polymerization or oligomerization¹⁷², or interactions dominated by strong hydrophobic effects or high-affinity binding that yield stable, kinetically trapped assemblies¹⁷³. Distinguishing these mechanisms from true phase separation requires rigorous assessment of saturation concentrations and the material properties of the assemblies; for further discussion see reference⁶².

Role of condensate–membrane interactions in vivo

Condensate–membrane interactions are ubiquitous across diverse organisms, including animals, plants, yeast, and bacteria¹⁷⁴⁻¹⁷⁶, mediating a myriad of essential cellular processes. Many observed behaviors in cells have been predicted by in vitro reconstitution experiments. Here, we highlight some of the best-understood processes through which membrane-bound and membraneless organelles integrate functionally.

Condensate–membrane interactions in cell signaling

Cell surface receptor activation frequently leads to the assembly of downstream signaling molecules into micro- or submicrometer-sized clusters¹⁷⁷. A hallmark example is the T-cell receptor (TCR) signaling system. Upon TCR activation, the transmembrane protein LAT undergoes phosphorylation, enabling it to recruit the cytoplasmic adaptors like Grb2 and Sos through multivalent interactions, forming a dynamic, liquid-like 2D condensate at the membrane surface^{17,18}. This reversible condensation creates a distinct biochemical compartment that selectively enriches kinases (e.g., the tyrosine-protein kinase ZAP70), while excluding phosphatases (e.g., the Receptor-type tyrosine-protein phosphatase CD45, also known as PTPRC), thereby sustaining LAT phosphorylation and boosting TCR signal strength and persistence^{6,17}. LAT condensates also promote downstream actin polymerization through the clustering of key actin regulators¹⁷. Crucially, LAT condensates couple with membrane lipid domains, spatially directing other signaling molecules like K-Ras⁶. K-Ras is targeted to these raft-like domains through post-translational lipidation, which allows it to partition into the ordered lipid environment. This reciprocal relationship, observed in live T cells where activation-induced Grb2 condensates colocalize with raft-like environments, is essential for effective signal transduction. This is demonstrated by replacing the native LAT transmembrane domain (TMD) by a nonraft-TMD, or replacing the Tyr residues by Ala, which prevents LAT phosphorylation and interaction with Grb2, leading to the uncoupling of LAT from raft domains and therefore abrogating both condensate formation and T cell activation⁶. Thus, protein condensates not only modulate signaling kinetics and compartmentalize biochemistry but also actively reorganize the membrane lipid landscape, providing a sophisticated mechanism for functional membrane organization in cell signaling⁶.

Prewetting in tight-junction formation

Tight junctions, crucial for epithelial cell–cell adhesion, paracellular permeability, and cell polarity^{121,178}, are formed by scaffold proteins called zonula occludens (ZO) proteins. ZO proteins assemble via LLPS into dense, dynamic membrane-attached compartments^{121,178-180}. Although ZO proteins possess inherent phase separation capacity, their cytoplasmic concentrations are typically below saturation¹⁷⁸, necessitating a localized mechanism enabling tight junction assembly. This is achieved through receptor-mediated prewetting: oligomerization of adhesion receptors like claudin-2 facilitates ZO-1 binding and co-condensation at cell–cell contact sites¹⁷⁹. Additionally, the apical polarity protein PATJ mediates preferential ZO-1 adsorption to the apical membrane, further promoting prewetting and tight-junction elongation¹²¹. This receptor-mediated surface condensation provides a robust, localized switch linking extracellular adhesion signals to intracellular scaffold assembly¹⁷⁹. The dynamic, liquid-like properties of these condensates allow for remodeling during junction assembly^{178,180}. Beyond prewetting, the active shaping of ZO-1 condensates into continuous belts is intricately linked to the actin cytoskeleton. High local ZO-1 concentration within condensates facilitates actin filament recruitment and polymerization, and F-actin dynamics can regulate ZO-1 localization, influencing cell–cell adhesion¹⁷⁸⁻¹⁸⁰.

Phase separation in presynapsis organization

Synaptic vesicle clusters, critical neurotransmitter reservoirs in presynaptic terminals, are dynamically organized by LLPS, rather than rigid scaffolds¹⁸¹. Synapsin-1 (Syn1), an abundant phosphoprotein, mediates synaptic vesicle clustering through LLPS⁸ (Figure 3d). In vitro, Syn1 condensates efficiently capture lipid vesicles, including native synaptic vesicles, maintaining a fluid-like organization with significant mobility of both vesicles and synapsin molecules^{8,182}. In vivo, Syn1 condensation actively dictates synaptic vesicle sequestration and dynamics within synaptic boutons (presynaptic nerve terminals), ensuring high mobility while confined¹⁸². This fluid organization facilitates efficient, piecemeal recruitment of synaptic vesicles to active zones during sustained activity, crucial for maintaining a dynamic reserve pool essential for robust neurotransmission^{8,182}. Synaptic vesicle clustering at the presynapse represents a complete wetting morphology, suggesting wetting transitions are key for neuronal communication.

Condensate–membrane interactions in stress and pathogen infection response

Stress granules, composed of RNA-binding proteins and RNA, assemble in response to various stresses¹⁸³. Stress granules associate with membrane-bound organelles¹⁸⁴ like lysosomes to facilitate their transport along microtubules toward perinuclear regions¹²⁴ (Figure 3d), and also critically protect against lysosomal membrane damage, which is common during cellular stress or pathogen activity¹⁰. Stress granules rapidly localize to and "plug" membrane ruptures on endolysosomes, stabilizing the damaged membrane¹⁰. This swift condensation, potentially triggered by local increases in acidity, prevents further leakage and enables membrane repair¹⁰ (see Figure 3d). In vitro GUV models confirm that protein condensates (e.g., Ras GTPase-activating protein-binding protein 1 (G3BP1)–RNA granules) can form at and wet membrane pores, stabilizing ruptures and promoting vesicle survival¹⁰. This universal condensate-mediated plugging mechanism for membrane damage stabilization, also observed at the nuclear membrane in a recent preprint¹⁸⁵, exemplifies how condensates exhibit emergent mesoscale properties beyond component molecular specificities.

Conclusions and perspectives

The intricate crosstalk between biomolecular condensates and cellular membranes has emerged as a fundamental organizing principle in cell biology, profoundly influencing membrane organization and remodeling, and a plethora of cellular functions. Recent insights highlight the crucial role of both membrane and condensate interfaces. Condensates affect membrane properties like lipid packing and hydration¹¹, mediate lipid demixing^{161,162}, and couple to specific domains^{6,126}. Moreover, condensates and membranes mutually reshape^{4,69,135,136} and stabilize each other¹⁰, highlighting their role in signalling^{6,17}, organelle remodeling¹²⁵ and morphogenesis⁷. Molecules in condensates can engage in selective interactions with specific lipids (e.g. phosphatidylinositol 4,5-bisphosphate (PIP₂) or phosphatidylinositol 3,4,5-trisphosphate (PIP₃)) leading to local lipid enrichment, and in some cases, inducing membrane phase separation that promotes signalling events. Confinement at the condensate–membrane interface can also reshape the local chemical microenvironment, enhancing biochemical activity, accelerating reactions and facilitating enzymatic cascades, including phosphorylation and other post-translational modifications. These mesoscale coupling effects illustrate how molecular properties of both scaffold proteins and lipids can dictate the emergent behavior of coupled 2D and 3D assemblies, highlighting a mechanistic layer that remains incompletely understood.

A remaining challenge is to fully comprehend how these diverse mechanisms orchestrate and interconnect, especially considering the often-overlooked influence of the cytoplasmic milieu, which necessitates characterizing both dense and dilute condensate phases^{79,105}. Addressing this complexity will require concerted efforts from both experimental and computational approaches. Achieving a holistic understanding demands combining technologies capable of probing properties across diverse spatiotemporal scales. Promising advances include hypotonic swelling to create large intracellular vesicles for high-resolution analysis of natural contact sites¹⁸⁶, along with expansion microscopy¹⁸⁷ and the use of giant organelle vesicles¹⁸⁸, invaluable for studying organelle interactions. Integrating advanced microscopy with spectroscopic techniques, such as hyperspectral imaging and fluorescence lifetime imaging microscopy (FLIM) with phasor analysis^{11,79,189,190}, yields rich information on interaction mechanisms and property changes. We foresee that combining these spectroscopic methods with super-resolution microscopy^{12,13} will enable precise measurement of condensate–membrane interactions in cells, providing important insights into their interplay in health and disease.

Glossary

WETTING: A process by which a biomolecular condensate spreads along or adheres to a membrane, analogous to a liquid droplet wetting a solid, but involving a soft, deformable lipid membrane.

RAFT: A transient, dynamic region within a cell membrane characterized by a distinct lipid and/or protein composition and altered physical properties (e.g., higher packing density, reduced fluidity) compared to the surrounding membrane.

PHASE DIAGRAM: A graphical representation that illustrates the stable physical states (phases) of a system as a function of parameters, like temperature, pressure, and/or composition.

SUPPORTED LIPID BILAYERS: Planar lipid bilayers assembled on solid substrates (for example, glass or mica) and often separated by a thin hydration layer. They are widely used as stable model membranes to investigate lipid and protein organization, dynamics, and phase behavior.

CRITICAL POINT: A specific thermodynamic state defined by variables such as temperature, composition, or interaction strength, at which the distinction between two coexisting phases (e.g., dense and dilute, or liquid-ordered and liquid-disordered) disappears and the phases become indistinguishable.

LINE TENSION: The interfacial energy per unit length at the boundary between two coexisting phases within a membrane, influencing domain shape, size, and dynamics; analogous to surface tension but acting along a line.

MEMBRANE SPONTANEOUS CURVATURE: An intrinsic property of a membrane, representing its preferred curvature in the absence of external forces; arises from molecular asymmetries (asymmetric lipid composition or protein binding), driving membrane remodeling like budding and tubulation.

MODULATED PHASE: A distinct state within phase-separated biological systems characterized by periodic or patterned spatial variations in composition or structure, often arising from competing molecular interactions.

GIANT UNILAMELLAR VESICLE (GUV): A single-bilayer lipid vesicle, typically 5-100 μm in diameter, widely used as a biomimetic model system to study membrane properties and functions.

GIBBS TRIANGLE: A triangular graphical representation depicting the phase behavior of a three-component system, where each vertex represents a pure component and points within the triangle represent different compositions.

SATURATION CONCENTRATION: The concentration above which a component (e.g., protein, polymer) undergoes phase separation in bulk, forming distinct coexisting phases. Below this, a homogeneous phase is observed.

BINODAL: A boundary curve on a phase diagram that separates a single-phase region from a two-phase region, indicating compositions where two distinct phases can coexist in equilibrium.

ATPS: An experimental system consisting of two immiscible aqueous polymer solutions (e.g., PEG and dextran) that spontaneously separate into two distinct liquid phases, commonly used as in vitro models for biomolecular phase separation.

MESOSCALE: Refers to an intermediate length scale (tens of nanometers to several micrometers) between molecular and macroscopic scales. In cell biology, this is where complex structures like organelle contact sites, membrane domains, and biomolecular condensates organize and interact.

OSTWALD RIPENING: A process of condensate or membrane domain coarsening whereby larger droplets or domains grow at the expense of smaller ones.

CONDENSATE AGING: A process where biomolecular condensates progressively lose their liquid-like properties, undergoing transitions to more viscous, gel-like, or solid states, often linked to altered function and pathologies like neurodegeneration.

PERMITTIVITY: A measure of a material's ability to polarize in response to an electric field. For example, water has high dielectric permittivity, screening electrostatic interactions, whereas oils have low permittivity, allowing strong electrostatic interactions.

MOLECULAR DYNAMICS SIMULATIONS: Computational methods that model the motion of atoms and molecules over time by numerically solving equations of motion, allowing the study of structure, dynamics, and interactions at atomic or coarse-grained resolution.

YOUNG–LAPLACE EQUATION: An equation describing the pressure difference across a curved interface as proportional to surface tension and interfacial curvature, governing the equilibrium shape of droplets.

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